PCT/GB2004/003088

GB04/003088

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 21 September 2004

Patents Form 1/77

Patents Act 1977
(Rule 16)

THE PATENT OFFICE
15 JUL 2003
RECEIVED BY HAND

The Patent Office

1/77

16JUL03 E822822-11 002890
P01/7700 0.00-0316553.7

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP9 1RH

1. Your reference — JWJ01059GB

2. Patent application number *(The Patent Office will fill in this part)* — 15 JUL 2003 — 0316553.7

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Daniel DENSHAM
36 Gabriel's Wharf
Water Lane
Exeter
EX2 4RN, United Kingdom

Patents ADP number *(if you know it)* — 8673501001

If the applicant is a corporate body, give the country/state of its incorporation — GB

4. Title of the invention — Method

5. Name of your agent *(if you have one)* — Gill Jennings & Every

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Broadgate House
7 Eldon Street
London
EC2M 7LH

Patents ADP number *(if you know it)* — 745002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	4
Claim(s)	- DL
Abstract	-
Drawing(s)	1 + 1

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	-
Request for preliminary examination and search *(Patents Form 9/77)*	-
Request for substantive examination *(Patents Form 10/77)*	-
Any other documents *(please specify)*	NO

11. For the applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature John Jappy Date 15 July 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

JAPPY, John William Graham
020 7377 1377

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

e) *Once you have filled in the form you must remember to sign and date it.*

f) *For details of the fee and ways to pay please contact the Patent Office.*

Method

Field of the Invention

The present invention relates to the field biosensors, and more specifically, concerned with methods for providing surfaces (preferably metal) with surface layers capable of selective biomolecular interactions whilst simultaneously enhancing single-to-noise ratio of the interaction event.

Background of the Invention.

There exists a large number of biosensors today within the art. According to Aizawa (1983) a biosensor is defined as being a unique combination of a receptor for molecular recognition, for example a selective layer with immobilized antibodies, and a transducer for transmitting the interaction information to processable signals. One such group of biosensors will detect the change which is caused in the optical properties of a surface layer due to the interaction of the receptor with the surrounding medium. Among such techniques may be mentioned especially ellipsometry and surface plasmon resonance.

In order for these types of techniques to work satisfactorily in actual practice certain requirements have to be fulfilled - i.e. the requirement that the sensing surface (or measuring surface) employed can be easily derivatized so that it will then contain the desired receptor, and moreover that it will not produce any (or only negligible) non-specific binding ,i.e. binding of components other than those that are intended. In somewhat simplified terms the technique of surface plasmon resonance - by abbreviation SPR, may be said to be a technique in which changes in the refractive index in a layer close to a thin metal film are detected by consequential changes in the intensity of a reflected light beam.

Particles of interest, such as proteins, may become inactivated upon binding to a flat surface and so a number of solutions have been employed within the art in order to reduce this limitation. One such solution is outlined within US patent no. 5436161, which is fully incorporated herein, in which a hydrogel matrix is bound to a surface and via which a desired ligand can be

bound. This hydrogel is activated to contain charged groups for bringing about the concentration of biomolecules carrying an opposite charge to that of said charged groups, and reactive groups for covalently binding the biomolecules concentrated to the matrix coating. This configuration has the advantage of increasing the amount of ligand bound whilst reducing non-specific interactions. These advantages also result, however, in a large proportion of the bound ligands binding at a distance from the metal surface of the biosensor. This will necessarily, therefore, reduce the binding signal size of the particle of interest as it binds to the dextran matrix. This is a result of the fact that the intensity of the plasmon field from a SPR supporting metal surface decrease as an inverse square as the distance away from the surface of the supporting layer increases.

As the demand for greater and greater sensitivity for measurements binding of small molecules increases, so does the need for a binding matrix that is not only compatible with proteins and other biomolecules and is capable or providing for covalent binding of such molecules, but also serves to increase sensitivity to such molecular interaction events. The present invention fulfills such requirements.

Summary of the Invention.

The present invention is based on the realization that the provision of a 3 dimensional matrix of plasmon supporting nanoparticles onto which biomolecules of interest may selectively bind will cause binding events to occur at high plasmon intensities resulting in enhanced energy/phase changes within the plasmon field encompassing said matrix.

According to one aspect of the present invention, a matrix suitable for use in a plasmon based biosensor which acts to enhance binding signals consisting of:

(i) Metallic particles capable of supporting surface plasmon modes whose surfaces are modified in order to facilitate binding of specific molecular species, being linked to further metallic particles via,

(ii) Polymer interconnects, connecting adjacent nanoparticles as well as binding said matrix to a surface.

In a preferred embodiment, the particles are gold nanoparticles in the size range 1 - 100 nm and these particles are modified to allow binding of molecular species via the immobilization thereon of short polymers attached to the gold surface via sulphide bond (thiol group).

In a preferred embodiment of the present invention, antibodies are immobilized on the particles surfaces in order to impact molecular specificity

In a further preferred embodiment of the current invention, the plasmon based sensor utilizes surface plasmon resonance.

In a further embodiment of the present invention the surface plasmon resonance sensor is based on measurements of changes in phase angle.

Description of the Drawings

Figure.1:

1 = plasmon supporting particle

2 = interconnecting polymer

3 = specialized interaction/immobilization layer

Description of the Invention

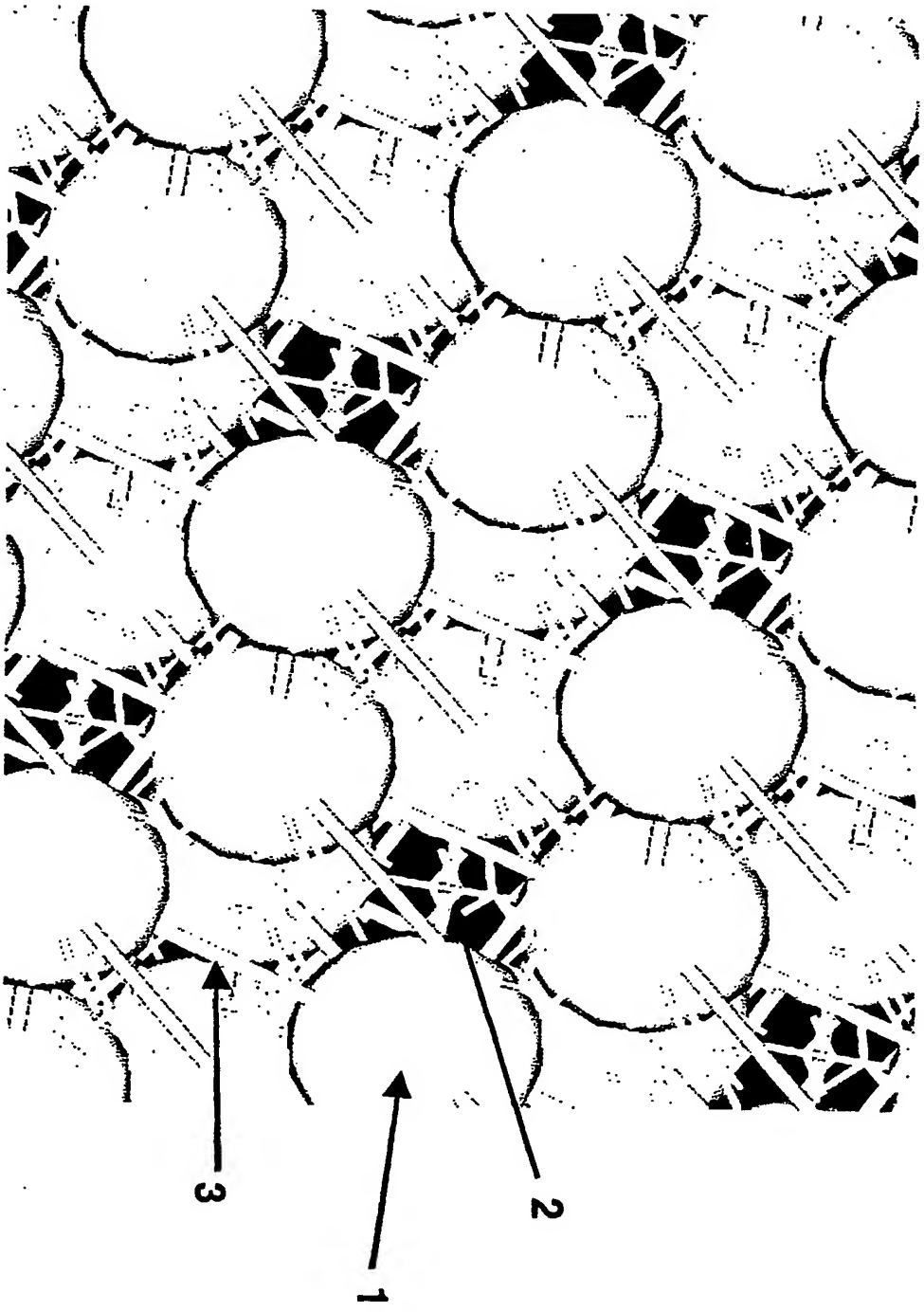
The present invention is based on the realization that the provision of a 3 dimensional matrix of plasmon supporting nanoparticles onto which biomolecules of interest may selectively bind will cause binding events to occur at high plasmon intensities resulting in enhanced energy/phase changes within the plasmon field encompassing said matrix.

The term "metallic particle" as used herein is to be interpreted broadly, and includes particle from 1 nm to several microns in size and which can be of any known shape and formed from any known plasmon supporting metal. Such metals include free electron metals such as e.g. copper, silver, aluminum or gold. The supporting surface is envisioned to consist of such a plasmon supporting metal (to satisfy the conditions for surface plasmon resonance (SPR)).

It is a preferred embodiment of the present invention that the plasmon supporting metal is gold, due to gold's superior corrosion stability.

Generally, initial modification of the gold surfaces (both the gold particles and that of the solid support) may be carried out in similar fashions. Generally, modification of gold surfaces with certain types of sulfur compounds has be described, for example by Nuzzo R.G. et al (1983), Porter M.D. et al (1987), and Troughton E.B. et al (1988).

A monolayer may be applied to the gold surface of the particle and the solid support using an applied molecular mono-layer, as is well known in the art. The simplest form of organic molecule for the formation of such a mono-layer can be represented as X-R-Y. It is preferred that the bonds being partially covalent in character; X binds to the metal and Y serves for coupling with functional ligands. This may be achieved when the ligand is coupled directly to Y or optionally after activation of Y. A number of techniques for such covalent modification are known in the art.


3
2
1